IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Bror Morein et al.
Appln. No.	:	10/550,026
Filed	:	June 11, 2007
Title	:	COMPOSITION COMPRISING ISCOM PARTICLES AND LIVE MICRO-ORGANISMS
Conf. No.	:	6185
TC/A.U.	:	1648
Examiner	:	Zachariah Lucas
Customer no.	:	00116
Docket No.:	:	ALBI-41848

DECLARATION UNDER 37 CFR 1.132

Sir:

This Declaration under 37 CFR 1.132 is filed in response to the outstanding Office action of August 6, 2009.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Bror Morein et al.
Appln. No. : 10/550,026
Filed : June 11, 2007
Title : COMPOSITION COMPRISING ISCOM PARTICLES AND LIVE MICRO-ORGANISMS

DECLARATION OF BROR MOREIN

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Bror Morein, having knowledge of the facts set forth herein, declares as follows:

1. Exhibits A and B are attached and are made part of this declaration.

2. I presently reside at Ollonstigen 3, Uppsala, Sweden.

3. I am a co-inventor of the subject matter claimed in the above-captioned patent application.

4. My qualifications, publication list, and record as an inventor are already of record based on my previously filed Declaration dated May 18, 2009.

5. I understand that the reference to Van Woensel (U.S. Pat. No. 5,925,359) remains at issue in this case. It is my opinion that Van Woensel lacks credibility with regard to any teaching, suggestion, or motivation that it may provide regarding methods or compositions including an adjuvant and a live micro-organism in a single composition for use as a

vaccine, because such methods or compositions would not have been expected to be desirable or useful and because the passages in Van Woensel cited by the Examiner provide no experimental evidence or other support to show any beneficial effect associated with an adjuvant incorporated into a live attenuated vaccine.

6. Such methods or compositions would not have been expected to be desirable or useful in view of the understanding in the art that an adjuvant is not necessary for generation of specific immunity for a live attenuated vaccine and that adjuvants are known to have deleterious effects on hosts.

7. This understanding is supported, for example, by a reference to H.F. Stills, Jr., 46 ILAR Journal 280, 280 (2005), submitted herewith, which provides insights regarding understanding in the art at the time of invention, and which states the following:

> Adjuvants have been used for more that 70 yr to enhance the immune response of the host animal to an antigen . . . . The immunostimulatory properties of adjuvants result in inflammation, tissue destruction, and the potential for pain and distress in the host animal . . . . Balancing the requisite degree of immunostimulation and the extent of inflammation, necrosis, and potential pain and distress requires consideration of the nature of the antigen, the host immune responsiveness, the adjuvant's mechanisms of action, and the desired end-product. In cases where the antigen is a weak immunogen or has a very limited availability, the type and role of adjuvant becomes a critical component in producing an acceptable immune response and humoral antibody response.

8. This understanding is also supported by various additional references to the European Medicines Agency and I.G. Barr et al., already of record.

9. Moreover, such methods or compositions would not have been expected to be desirable or useful in view of the knowledge in the art that various saponins have antimicrobial activities and thus would be expected to have deleterious effects on a live micro-organism, particularly an attenuated micro-organism, if included therewith in a single composition. This understanding is supported by various additional references to Sparg et al. and Francis et al., already of record, as well as statements in my previously filed Declaration dated May 18, 2009.

10. I understand that the reference to Morein (U.S. Pat. No. 5,679,354), of which I am a co-inventor, also remains at issue in this case. In my opinion, there is nothing in the Morein reference to suggest that live viruses were intended or would be useful. Indeed, one of ordinary skill would have recognized that the viruses expressly disclosed in the cited passage, i.e. picornavirus, adenovirus, and parvovirus, are virulent viruses, and given the absence of a qualification regarding attenuation would have recognized that the Morein reference was referring to killed virus. For comparison, see Example 4 of the Specification of the present Application, which refers to a commercial live vaccine and notes that it is a live attenuated vaccine against Canine Distemper, Adeno, Parvo and Parainfluenza virus. Also, as I have previously averred, when the inventors of the '354 patent, including myself, indicated in the '354 patent that iscom matrix could be used as an adjuvant with whole organisms, we did not intend that Quillaja saponin and/or iscom matrix/iscom particles would be used with <u>live</u> whole microorganisms.

11. Assuming, for purposes of argument only, that one of ordinary skill would have been motivated to combine Van Woensel and Morein to achieve the method and composition as claimed in claims 1 and 9, it is my opinion that one of ordinary skill would not have had a reasonable expectation of success with respect to achieving the method or composition of claims 1 or 9 for the intended purpose of use as a vaccine, for at least the reason that iscom particles were known to trigger multiple, potentially deleterious, and poorly characterized inflammatory responses in a host, and the extent and effects thereof upon administration of an iscom particle and a live micro-organism in a single composition would not have been predictable.

12. In this regard, it was known in the art that iscom particles trigger multiple inflammatory responses in a host. The fact that iscom particles were known to trigger multiple inflammatory responses is shown, for example, in a reference to R.E. Smith et al., 162 Journal of Immunology 5536, 5536 (1999), which is submitted herewith and which states with regard to experiments in mice involving injection of iscoms including OVA protein that "[t]he i.p. injection of ISCOMS induced intense local inflammation, with early recruitment of neutrophils and mast cells followed by macrophages, dendritic cells, and lymphocytes." The reference elaborates that although iscoms trigger multiple responses, only production of IL-12 appears to be of major importance with regard to generating antigen-specific immunity, stating the following:

> The results presented here indicate that ISCOMS induce intense local activation of the innate immune response, recruiting a wide variety of inflammatory cells, including neutrophils, mast cells, DC, Mϕ, and lymphocytes. Many of these cells are activated, as evidenced by the expression of surface activation

> markers and the production of cytokines and other mediators. However, the majority of these factors were not essential for the immunogenicity of ISCOMS in vivo, with only the production of immunoreactive IL-12 appearing to be of major importance.

Id. at 5541-42. Of note, this passage suggests that the non-IL-12 inflammatory responses induced by iscom particles may be extraneous based on being nonessential.

13. It was of course also known that inflammation can be highly detrimental to a host and to a live micro-organism therein, for example because inflammation can cause pain to the host and because inflammation is in part aimed at removing from the host an injurious stimuli such as the live micro-organism. Indeed, attenuated micro-organisms such as micro-organisms included in a live vaccine would be expected to be particularly susceptible to inflammation, as attenuated micro-organisms are specifically weakened with regard to their ability to maintain infection and thus are particularly susceptible to being killed, as demonstrated for example in a reference to a Nobivac Tricat Data Sheet, http://www.intervet.co.uk/Products_Public/Nobivac_Tricat/090_Product_Datasheet.asp, submitted herewith as Exhibit A, which highlights the extent to which the attenuated viruses in a live attenuated vaccine, specifically feline viral rhinotracheitis virus, feline calicivirus, and feline panleucopenia virus, are susceptible to killing. Specifically, the Nobivac Tricat Data Sheet states the following:

> The contents of one vial of reconstituted vaccine should be injected subcutaneously. Reconstitute immediately prior to use by the addition of the contents of one vial Nobivac Solvent, Nobivac FeLV or Nobivac Rabies. Sterile

> equipment should be used for administration. Avoid contamination of vaccine with traces of chemical sterilising agents. Do not use chemicals such as disinfectant or spiril to disinfect the skin prior to inoculation.

Id. The statements that the vaccine should be reconstituted immediately prior to use and that contamination with even traces of chemical sterilising agents should be avoided highlight the susceptibility of the live attenuated viruses to being killed, e.g. by prolonged storage after reconstitution and/or by chemicals. The above-noted iscom-mediated non-IL-12 inflammatory responses would have been of particular concern, given their risk of harming the host and the live micro-organism therein, with no expected concomitant benefit in terms of providing specific immunity.

14. The bases of iscom-triggered multiple inflammatory responses in general, and iscom-triggered IL-12 production in particular, were not understood. This is apparent, for example, from the Smith reference, which concludes as follows:

> [O]ur results show that the ability of ISCOMS to induce a wide range of Ag-specific immune responses [i.e. antigen-specific immune responses] is paralleled by the activation of a cascade of innate immune responses. This is consistent with other evidence that the best adjuvants are those that mimic the ability of pathogens to activate the innate immune system. However, our study also reveals the complexity of the resulting non-specific signals that are generated, with many overlapping and redundant mechanisms employed, only a few of which may play an essential role in the development of an Ag-specific immune response. Elucidating and targeting these mechanisms will be important in the

design of a successful vaccine.

Id. at 5545. This is also evident from Smith's statement that "[t]he source of ISCOMS-induced IL-12 remains to be determined." *Id.* at 5544.

15. It is my opinion that because iscom particles were known to trigger multiple inflammatory responses, the responses were known to be potentially highly detrimental, and the bases of these iscom-mediated inflammatory responses were not understood, one of ordinary skill would not have had a reasonable expectation of success regarding achieving a method or composition including an iscom particle and a live micro-organism in a single composition, as claimed in claims 1 and 9. Put another way, although a live micro-organism and an iscom particle were both known to contribute to generation of specific immunity in different contexts, the result of combining the two in a single composition for use as a vaccine was not reasonably predictable. By way of illustration, starting at a high degree of specificity, IL-12 was understood to be important not just with regard to iscom-mediated adjuvation, as indicated above, but also with regard to infection of hosts by intracellular bacteria and with regard to tissue damage based on excessive inflammation. The latter two points are shown, for example, in a reference to Y. Zhan et al., 161 Journal of Immunology 1447, 1447 (1998), which is submitted herewith and which states that "[o]ne of the early events in infection with intracellular bacteria is phagocytosis by resident macrophages and the release of cytokines by these cells," and that "[a] key role is played by IL-12, which up-regulates the production of IFN-γ by T cells and NK cells." Zhan teaches that i.v. infection of mice with the intracellular pathogen *Listeria monocytogenes* or the vaccine strain 19 of *Brucella abortus* results in detectable production of bioactive IL-12 followed by cessation of

production of IL-12 while bacteria still persisted in high numbers. *See id.* at 1447-48, 1451. Zhan also teaches that "[t]hese experiments show that, in vivo, live but not killed *Listeria* and *Brucella* organisms triggered the secretion of detectable IL-12 bioactive protein" *Id.* at 1450. Zhan also notes that "[i]t has been said that IL-12 being a strongly inflammatory cytokine must be down-regulated before tissue damage occurs." *Id.* at 1451. Accordingly, Zhan, in view of Smith, suggests that careful control of IL-12, not just in terms of production but also in terms of cessation of production, would have been a crucial factor for achieving success in the development and use of a vaccine including an iscom particle and live micro-organism. However, it would not have been apparent from these references whether the effect of an iscom particle and a live micro-organism on IL-12 production would have been e.g., additive, synergistic, less than the sum of the parts, or something else entirely, and further the effect of the resulting IL-12 production on the host and the live micro-organism therein would also not have been apparent. It also would not have been apparent from these references whether and to what extent IL-12 levels might become excessively high based on induction by both an iscom particle and live micro-organism, and/or whether and to what extent cessation of production of IL-12 might be affected, and again how this might affect the host and live micro-organism therein. Accordingly, there would not have been any degree of predictability regarding how administration of a single composition including both an iscom particle and an intracellular bacterium might affect IL-12 production, and thus the desirability and usefulness of the composition as a vaccine also would not have been reasonably predictable.

16. Now considering the issue more broadly, this concern would also have been applicable regarding not just

intracellular bacteria but other micro-organisms too because induction of IL-12 production had been shown to be important for pathogenicity in other micro-organisms, as shown for example in a reference to M.S. Di Genaro, 71 Infection & Immunity 1804, 1804-05 (2003), which is submitted herewith and which indicates that IL-12 is essential for clearance of infections of *Yersinia enterocolitica*, a gram-negative, extracellularly located pathogen. The above-noted lack of predictability would also have been compounded upon consideration of the above-noted fact that iscom particles were also known to induce multiple additional and potentially deleterious inflammatory responses, the bases of each of which, again as indicated above, were not understood.

17. Assuming, for purposes of argument only, that one of ordinary skill would have been motivated to combine Van Woensel and Morein and would have had a reasonable expectation of success, it is my opinion that the method and composition as claimed in claims 1 and 9 would still have been non-obvious because the use of a composition including an iscom particle and live micro-organism as a vaccine provides a greater than expected result in terms of not decreasing replication of the micro-organism and in terms of increasing antibody titer against the live micro-organism.

18. In this regard, arguments and supporting evidence are provided in the Examples 2-4 and Tables 2-4 of the Specification as filed.

19. Specifically, as explained in Example 2 of the Specification and as supported by experimental evidence, my Co-Inventor and I have demonstrated that the method and composition of claims 1 and 9, including an iscom particle and a live micro-organism in a single composition, provide a greater than expected result in terms of not decreasing

replication of a live micro-organism, based on experimental data that none of the iscom and iscom matrix formulations tested reduced the EID50 titres [i.e. the end point where 50% of the embryos are infected] compared to the control groups, and that in contrast oil and aluminum hydroxide reduced the EID50 titres more than a 10 log. The results were greater than expected, for at least the reasons that iscom particles include saponins and various saponins were known to have anti-microbial activities, as discussed above, and thus it would have been expected that mixing iscom particles and the influenza virus in a single composition would have resulted in a decreasing replication of the live micro-organism relative to the absence of iscom particles.

20. Moreover, as explained in Example 3 of the Specification and as supported by experimental evidence, my Co-Inventor and I have demonstrated that the method and composition of claims 1 and 9, including an iscom particle and a live micro-organism in a single composition, provide a greater than expected result in terms of not decreasing replication of a live micro-organism, based on experimental data that A-matrix, C-matrix treated virus titered both out to 5.7 i.e. a ten fold higher titre than the virus control i.e. an un-expected increase in virus growth. Moreover, my Co-Inventor and I have demonstrated that 703 matrix (4.7), A+C-matrix (4.7), Q-VAC matrix (4.5), free saponin A, influenza virus Iscoms (4.9) and bovine respiratory syncytial virus Iscoms (4.4) exhibited titres that did not significantly differ from the titres of the virus control. In contrast, Spikoside matrix, free saponin C, free 703 and free spikoside, oil adjuvant and aluminum hydroxide decreased more than ten fold the virus titres compared to the virus control. The results were greater than expected, for the reasons indicated above regarding Example 2.

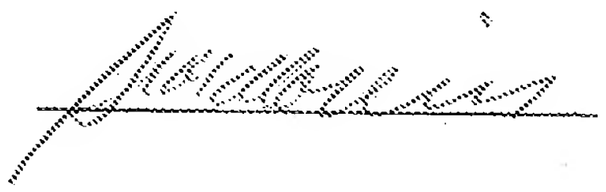
21. In addition, as explained in Example 4 and Tables 2-4 of the Specification and as supported by experimental evidence, my Co-Inventor and I have demonstrated that the method and composition of claims 1 and 9, including an iscom particle and a live micro-organism in a single composition, also provide a greater than expected result in terms of increasing titres of antibodies to live vaccine components included in a vaccine, based on experimental data that serum antibody responses were higher against both live antigens and against the killed rabies virus vaccine antigen in the animals immunized with vaccine supplemented with MM703 and MB703 formulations than in the control group that was immunized with non-adjuvanted vaccine. Assuming for purposes of argument only that one of ordinary skill would have had a reasonable expectation of success with regard to use as a vaccine, the results were greater than expected given that, as indicated above, attenuated viruses were known to be particularly susceptible to being killed, e.g. by chemicals, iscom particles include saponins, various saponins are known to have anti-microbial activities, but nonetheless the iscom matrix preparations did not apparently harm the attenuated viruses but rather led to an increase in their effectiveness at triggering the generation of antibodies. The results were also greater than expected in view of the understanding in the art, as discussed above, that an adjuvant is not necessary for generation of specific immunity for a live attenuated vaccine and that adjuvants are known to have deleterious effects on hosts.

22. Moreover, additional supporting evidence is provided here, as follows. A greater than expected result in terms of increasing titres of antibodies to live vaccine components included in a vaccine was observed with respect to additional live viruses in an additional host animal. Specifically, we

have demonstrated with experimental data that the antibody responses were higher in the animals immunized with vaccine supplemented with iscom matrix MM703 including 83% of Fraction A and 17% of Fraction C of Quil A than in the control group that was immunized with non-adjuvanted vaccine, as shown in Exhibit B. In the experiments we tested antibody titres based on vaccination of cats once with Nobivac Tricat formulation, a live attenuated vaccine including feline viral rhinotracheitis virus, feline calicivirus, and feline paneleucopenia virus, as described in Exhibit A, and had observed, upon measuring antibody titres at fourteen days after vaccination, that for all three viruses tested the antibody response was higher when the live attenuated viruses were administered together with iscom matrix. Again, for the reasons indicated above regarding Example 4, the results were greater than expected.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.

Inventor Name: Bror Morein

Signature: 

Country of Citizenship: Sweden

Address: Ollonstigen 3 SE 75591 Uppsala, Sweden

Date: 4/11 - 2009

Intervet

Search...

Companion Pets Equines Livestock Poultry Vets SOPs

Nobivac Tricat

Overview

Packsizes

Support Materials

Product Data Sheet

Product class
Vaccine - Cat

Nobivac Tricat Data Sheet

Freeze-dried pellet for suspension for injection for cats. Presentation
Live vaccine containing per dose of 1 ml $\geq 10^{6.2}$ $TCID_{50}$ Feline viral rhinotracheitis virus; $\geq 10^{4.7}$ pfu Feline calicivirus; $\geq 10^{4.2}$ $TCID_{50}$ Feline panleucopenia virus.

Uses
For the active immunisation of cats to reduce clinical signs of disease following infection with feline viral rhinotracheitis virus (feline herpesvirus) and/or feline calicivirus. To prevent clinical signs of disease and leucopenia following infection with feline panleucopenia virus, and to reduce excretion of FHV and virulent FPLV.

Specific claims:
An onset of immunity of 7 days after primary vaccination has been demonstrated for the feline calicivirus, feline rhinotracheitis virus and feline panleucopenia virus components.

Dosage and administration
The contents of one vial of reconstituted vaccine should be injected subcutaneously. Reconstitute immediately prior to use by the addition of the contents of one vial Nobivac Solvent, Nobivac FeLV or Nobivac Rabies. Sterile equipment should be used for administration. Avoid contamination of vaccine with traces of chemical sterilising agents. Do not use chemicals such as disinfectant or spirit to disinfect the skin prior to inoculation.

Primary course vaccination:
For the primary course vaccination, 2 doses are required with an interval of 3 to 4 weeks between vaccinations. The first dose may be administered to kittens from 9 weeks of age.

Revaccination scheme:
To maintain protection a single annual booster dose is recommended.

Further information:
Experience has shown that the maternal antibody status of kittens within a litter varies greatly and reliance should not be placed on serological examination of the queen alone.
A good immune response is reliant on the reaction of an immunogenic agent and a fully competent immune system. Immunogenicity of the vaccine antigen will be reduced by poor storage or inappropriate administration. Immunocompetence of the animal may be compromised by a variety of factors including poor health, nutritional status, genetic factors, concurrent drug therapy and stress.

Contra-indications, warnings, etc.
Only healthy animals should be vaccinated.
The vaccine may not be effective in cats incubating the disease at the time of vaccination.

A slight transient swelling (<5mm) may be observed at the site of injection for one day.
Where Nobivac FeLV has been used to reconstitute Nobivac Tricat prior to inoculation, a small, painless nodule at the site of vaccination is frequently observed. It can persist for up to 13 days post inoculation.
In the rare event of a hypersensitivity reaction following vaccination, administer an antihistamine, corticosteroid or adrenaline, without delay and by the most immediate route.
After administration of an overdose a transient swelling (<5mm) at the injection site may occur for six days. A transient increase in temperature and some lethargy may occur for one day.
No specific treatment or antidote is recommended.

Care should be taken to ensure correct systemic administration of the vaccine. Inadvertent nasal or oral dosing (e.g. by making an aerosol with the syringe, or the cat licking the injection site) may result in clinical signs of respiratory disease including lethargy and malaise.
Swabbing the injection site with spirit *after* vaccination is a useful precaution.
Some animals may be immunologically incompetent and fail to respond to vaccination. Animals that have received the corresponding anti-serum or immunosuppressive drugs should not be vaccinated until an interval of at least 4 weeks has elapsed.

Do not use in pregnant animals, as this has not been tested.

No information is available on the safety and efficacy from simultaneous use of Nobivac Tricat with any other vaccine except Nobivac FeLV and Nobivac Rabies. It is therefore recommended that no other immunological product should be administered within 14 days before or after vaccination with Nobivac Tricat.
Do not mix with any other medicinalproduct other than listed above.

The efficacy of the FCV, FVR and FPLV components of the vaccine may be reduced due to maternal antibody interference. However, the vaccine has been proved to be of benefit against virulent challenge in the presence of maternal antibody levels to FCV, FVR and FPLV that are likely to be encountered under field conditions.

Withdrawal period:
Not applicable.

FOR ANIMAL TREATMENT ONLY. KEEP OUT OF REACH AND SIGHT OF CHILDREN.

Pharmaceutical precautions
Store and transport between +2°C and + 8°C. Care should be taken to avoid prolonged or repetitive exposure to high ambient temperatures following withdrawal from the refrigerator prior to use - in hot summer conditions vaccine potency can be severely reduced within a few hours.
After reconstitution: use within 30 minutes.

Disposal advice:
Dispose of waste material by boiling, incineration or immersion in an appropriate disinfectant approved for use by the competent authorities.

Legal category
POM-V To be supplied only on veterinary prescription.

Package quantities
Clear glass Type I (Ph.Eur.) single dose vials with halogenobutyl rubber stopper, closed with a colour-coded aluminium cap. Cartons containing 5, 10, 25 or 50 vials and can be presented either with the diluent or without. Not all presentations may be marketed.

Further information
Nil.

Marketing Authorisation number
Vm 01708/4514 UK authorised veterinary medicinal product.

Marketing Authorisation holder
Intervet UK Ltd.
Walton Manor
Walton
Milton Keynes
Bucks, MK7 7AJ

Distributed in Northern Ireland by:
Intervet Ireland Ltd.
Magna Drive
Magna Business Park
Citywest Road
Dublin 24

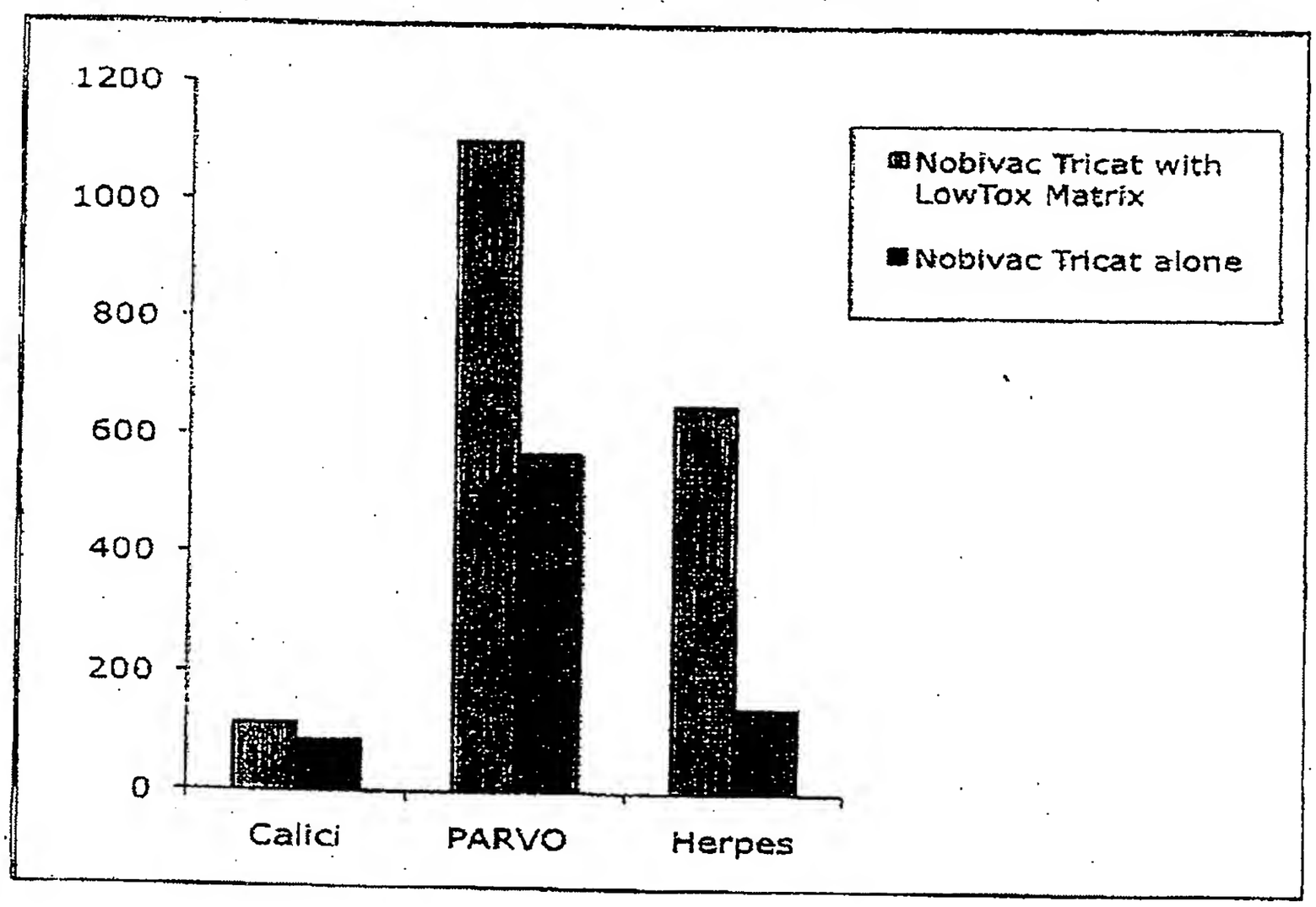
Date of text preparation
17/05/06

Exhibit B

Example Live attenuated cat vaccine with or without Matrix addition

Serum samples were analysed for antibody response to the different live attenuated viruses 14 days after vaccination.

Cats were vaccinated once with Nobivac Tricat with or without addition with ISCOM Matrix according to the manufacturer's instructions. The ISCOM matrix comprised of 83% of fraction A and 17 % of fraction C of Quil A.



For all three viruses tested the antibody response was higher when the live attenuated viruses were administrated together with ISCOM matrix.